LECTURE

The 2004 Aschoff/Pittendrigh Lecture: Theory of the Origin of the Pineal Gland—A Tale of Conflict and Resolution

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Abstract A theory is presented that explains the evolution of the pinealocyte from the common ancestral photoreceptor of both the pinealocyte and retinal photoreceptor. Central to the hypothesis is the previously unrecognized conflict between the two chemistries that define these cells—melatonin synthesis and retinoid recycling. At the core of the conflict is the formation of adducts composed of two molecules of retinaldehyde and one molecule of serotonin, analogous to formation in the retina of the toxic *bis*-retinyl ethanolamine (A2E). The hypothesis argues that early in chordate evolution, at a point before the genes required for melatonin synthesis were acquired, retinaldehyde—which is essential for photon capture—was depleted by reacting with naturally occurring arylalkylamines (tyramine, serotonin, tryptamine, phenylethylamine) and xenobiotic arylalkylamines. This generated toxic *bis*-retinyl arylalkylamines (A2AAs). The acquisition of arylalkylamine *N*-acetyltransferase (AANAT) prevented this by *N*-acetylating the arylalkylamines. Hydroxyindole-O-methyltransferase enhanced detoxification in the primitive photoreceptor by increasing the lipid solubility of serotonin and *bis*-retinyl serotonin. After the serotonin → melatonin pathway was established, the next step leading toward the pinealocyte was the evolution of a daily rhythm in melatonin and the capacity to recognize it as a signal of darkness. The shift in melatonin from metabolic garbage to information developed a pressure to improve the reliability of the melatonin signal, which in turn led to higher levels of serotonin in the photodetector. This generated the conflict between serotonin and retinaldehyde, which was resolved by the cellular segregation of the two chemistries. The result, in primates, is a pineal gland that does not detect light and a retinal photodetector that does not make melatonin. High levels of AANAT in the latter tissue might serve the same function AANAT had when first acquired—prevention of A2AA formation.

Key words pinealocyte, retinal photoreceptor, melatonin synthesis, retinoid recycling, serotonin → melatonin pathway

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JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 19 No. 4, August 2004 264-279
DOI: 10.1177/0748730404267340

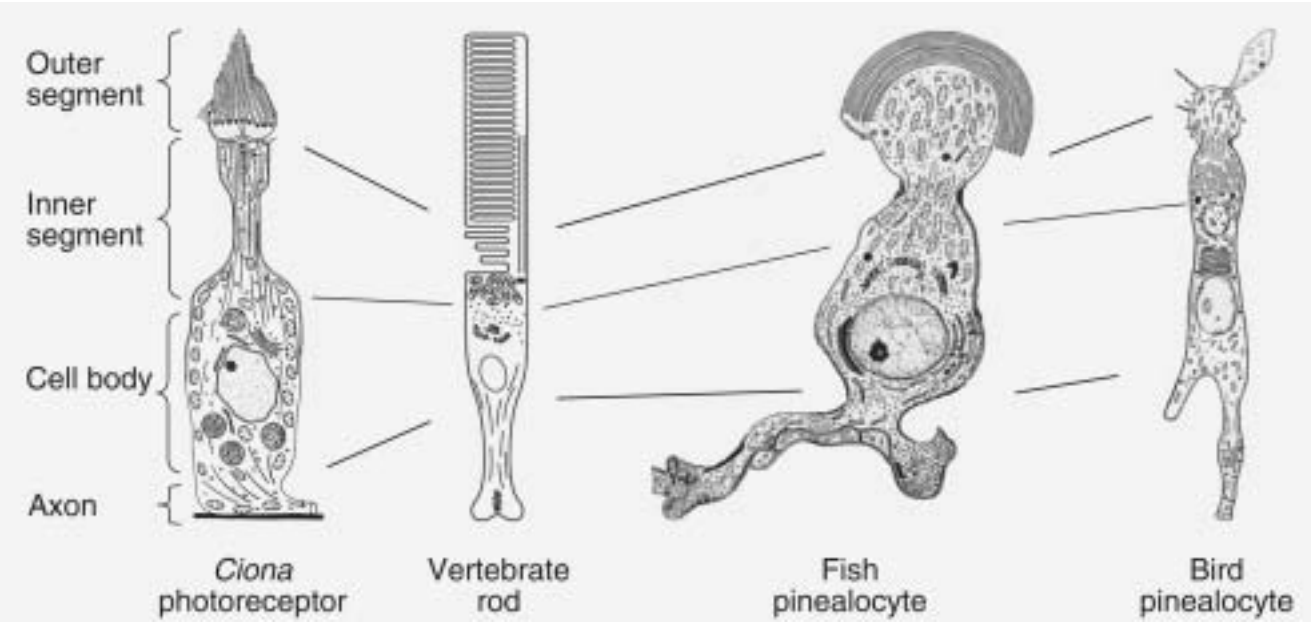

Figure 1. Photoreceptor cells of the urochordate *Ciona intestinalis*, vertebrate rod, fish pinealocyte, and bird pinealocyte. Based on drawings in the literature (Eakin, 1973; Collin, 1971).

Why a pinealocyte?
How did the pinealocyte evolve?
What selective pressures directed the evolution of melatonin as a signal of nighttime?
Why is the mammalian pinealocyte not sensitive to light?

These issues have received little attention in the literature, in contrast to the significant interest in the evolution of the pineal gland and pineal complexes and their similarity to retinal photoreceptors. This has generated a rich and fascinating literature that is of central importance to the focus of the theory of pineal evolution detailed here because it established that the pinealocyte and retinal photoreceptor had a common cellular origin.

This literature has been well reviewed, and those interested in learning more about it, about the remarkable diversity of pineal cytoarchitecture and the genetic similarity of pineal and retinal photoreceptors (Fig. 1), are encouraged to start by first reading the collection of articles assembled by J. Ariens Kappers and J. P. Schadé (1965) and *The Third Eye* by Richard M. Eakin (1973). Both will reward one with content; the latter will also provide an insight into the thinking of a pioneer who was among the first to use the electron microscope for comparative analysis of photoreceptor structures. One can then move on to the classic anatomical works of Andreas Oksche (1965, 1971, 1983, 1984), those by Jean Pierre Collin (1968, 1971, 1977), and their collaborative effort (Collin and Oksche, 1981). The issue of pineal-retinal relationships and similarities is also covered in a collection published in 1986 (O'Brien and Klein, 1986). More recent developments on the structure and function of the pineal gland are reviewed by Jack Falcon (1999), Peter Ekstrom and Hilmar Meissl (2003), Horst Korf (1994, 1999), and my group (Ganguly et al., 2002; Klein et al., 1997, 1998, 2002). These detail the diversity of the cells that compose vertebrate pineal glands and how they are organized and function; consideration is also given to evolutionary trends. A 1991 review by Andres Oksche is of special interest because it outlines the intellectual advances leading to the concept of photoneuroendocrinology and how this impacted circadian biology (Oksche, 1991).

The next wave of advances was focused on the molecular similarities of pinealocytes and retinal photoreceptors. One of the central messages coming from the molecular approach is that the retinal photoreceptor and pinealocyte, to a large degree, express the same sets of phototransduction and melatonin synthesis genes; this generality appears to apply to submammals, whose retinae and pinealocytes both detect light and make melatonin. However, it does not apply to mammals because genes required for photodetection (opsin and transducin alpha) have not been found to a significant degree in the mamma-

lian pinealocyte, and some required for melatonin production (HIOMT) do not appear to be expressed at a significant degree in the mammalian retina.

The group of ~20 phototransduction genes was first identified by retinal scientists; some were subsequently identified in pinealocytes, including transducin (van Veen et al., 1986a, 1986b; Babila et al., 1992), arrestin (S-antigen; Kalsow and Wacker, 1977; Donoso et al., 1985; Korf et al., 1985; Collin et al., 1986a, 1986b), phosducin (MEKA; Reig et al., 1990; Schaad et al., 1991), recoverin (Korf et al., 1992; Bastianelli and Pochet, 1994), opsin kinase (Somers and Klein, 1984; Ho et al., 1986), and interphotoreceptor retinoid binding protein (Rodrigues et al., 1986). There also has been notable evolutionary innovation leading to pineal-specific opsins in photosensitive pinealocytes (Okano et al., 1994; Max et al., 1995; Blackshaw and Snyder, 1997; Mano et al., 1999).

The role of phototransduction genes in the nonphotosensitive pinealocyte seems highly likely to be similar to that in the retina-signal transduction. In the case of the mammalian pinealocyte, this is likely to represent a dedication to adrenergic signal transduction rather than phototransduction because this cell is regulated by norepinephrine. The homologs of phototransduction genes (receptor kinases, producinlike proteins and arrestins), which evolved to function in signal transduction in brain and other tissues, are absent from the pineal gland, which emphasizes the close pinealocyte-retinal photoreceptor link—apparently the pinealocyte retained the original cast of phototransduction characters.

The evidence that the retina has the capacity to synthesize melatonin was accumulated first by scientists working on the pineal gland, who found one of the melatonin synthesizing enzymes present in the retina (Axelrod et al., 1965); subsequently, the fish, frog and bird retinae, and, to a lesser degree, the rodent retina were found to make melatonin, albeit at levels significantly lower than the pineal gland (Falcon, 1984; Falcon et al., 2003; Flight et al., 1983; Cahill et al., 1991; Iuvone et al., 1999; Tosini and Fukuhara, 2003); primates appear to lack this ability (Coon et al., 2002). There is little evidence that retinal melatonin contributes significantly to circulating melatonin; rather, it is thought to play a local paracrine role.

Another body of evidence indicating that the pineal gland and retina are closely linked come from the finding of transient photoreceptor-like elements in developing pinealocytes (Zimmerman and Tso, 1975) and the demonstration that the expression of photoreceptor features in pinealocytes can be experimentally manipulated in vitro (Araki, 1992; Araki and Tokunaga, 1990; Araki et al., 1988, 1992). These and other findings have provided the basis of a provocative consideration of the relative importance of the evolution of pinealocytes versus the evolution of developmental influences in determining pinealocyte features in vertebrates (Ekstrom and Meissl, 2003).

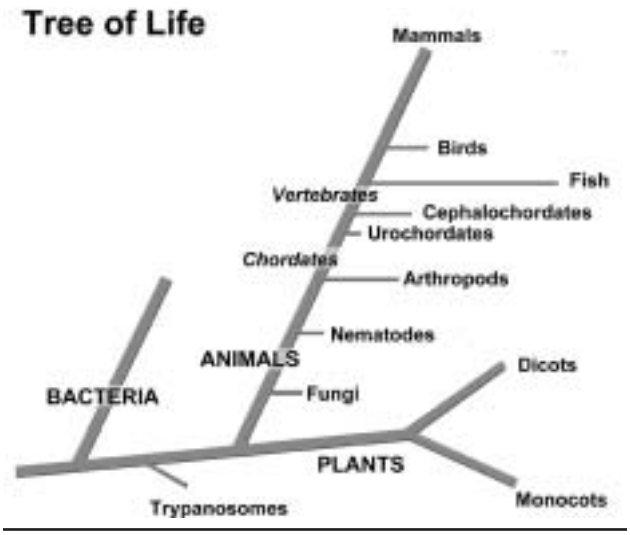

Figure 2. The Tree of Life.

The most recent wave of evidence linking the pineal gland and retina comes from analysis of transcription factors, which appear to act through similar or identical binding sites to control the developmental expression of genes in both tissues. Significant work has been done on members of the CRX/OTX family (Chen et al., 1997; Furukawa et al., 1999; Bernard et al., 2001; Gamse et al., 2002; Nishida et al., 2003; Appelbaum et al., 2004).

Against this background of anatomical and molecular evidence pointing to a common ancestor of the pinealocyte and retinal photoreceptor, we can ask: What determined the evolutionary vector from photoreceptor to pinealocyte?

OUTLINE OF THE "CONFLICT AND RESOLUTION" HYPOTHESIS OF PINEAL EVOLUTION

The following pages detail for the first time a hypothesis that describes the evolutionary transition of photoreceptor to pinealocyte. The story will take readers on new thought-provoking paths into unfa-

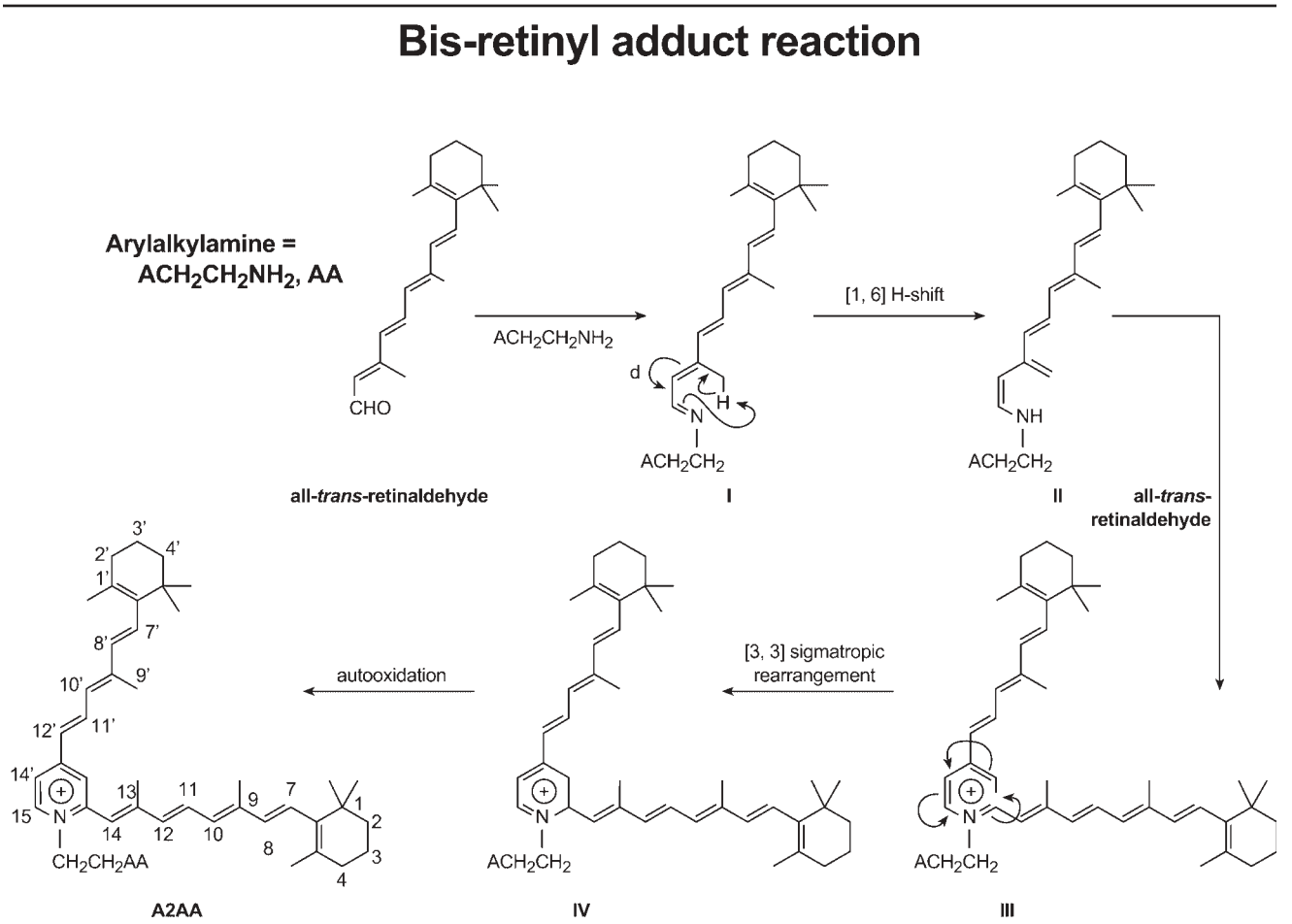

Figure 3. Formation of *bis*-retinyl arylalkylamines (A2AAs) based on the formation of *bis*-retinyl ethanolamine (Sparrow, 2003). Formation of the Schiff base intermediate (compound II) and final product of the reaction has been confirmed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) using four arylalkylamines (tryptamine, tyramine, phenylethylamine, serotonin) (SL Coon and DC Klein, unpublished).

miliar intellectual territory. Accordingly, it seems wise to provide a brief outline—a preview of the journey.

The hypothesis starts at a time in chordate evolution that preceded the emergence of vertebrates (Fig. 2), when the genes required for melatonin synthesis and signal transduction were absent from the chordate lineage. At the core of the hypothesis is a previously unrecognized chemical incompatibility—a conflict—between arylalkylamines and retinaldehyde (Figs. 3 and 4). Retinaldehyde is the vitamin A derivative critical for vertebrate photodetection—one molecule of *cis*-retinaldehyde is used to capture one photon of light. Arylalkylamines are generated endogenously by decarboxylation of aromatic amino acids (tryptophan → tryptamine, phenylalanine → phenylethylamine, tyrosine → tyramine, hydroxytryptophan → serotonin; Fig. 4); they are also of environmental origin—xenobiotics.

The acquisition of AANAT is the critical first step toward the evolution of melatonin as a hormone, which also required acquisition of hydroxyindole-O-methyltransferase (HIOMT), assembly of a melatonin factory, appearance of a night/day rhythm in melatonin production, and downstream melatonin signal transduction.

Arylalkylamines and retinaldehyde react nonenzymatically to form adducts in which two molecules of retinaldehyde are linked via a stable bond to one molecule of arylalkylamine—hence the name *bis*-retinyl arylalkylamine (A2AA) (Fig. 3). According to this hypothesis, A2AA adduct formation is a strong negative factor selecting against survivability because it depletes retinaldehyde, thereby eroding visual sensitivity; in addition, A2AAs are toxic—hence the retinaldehyde/arylalkylamine conflict.

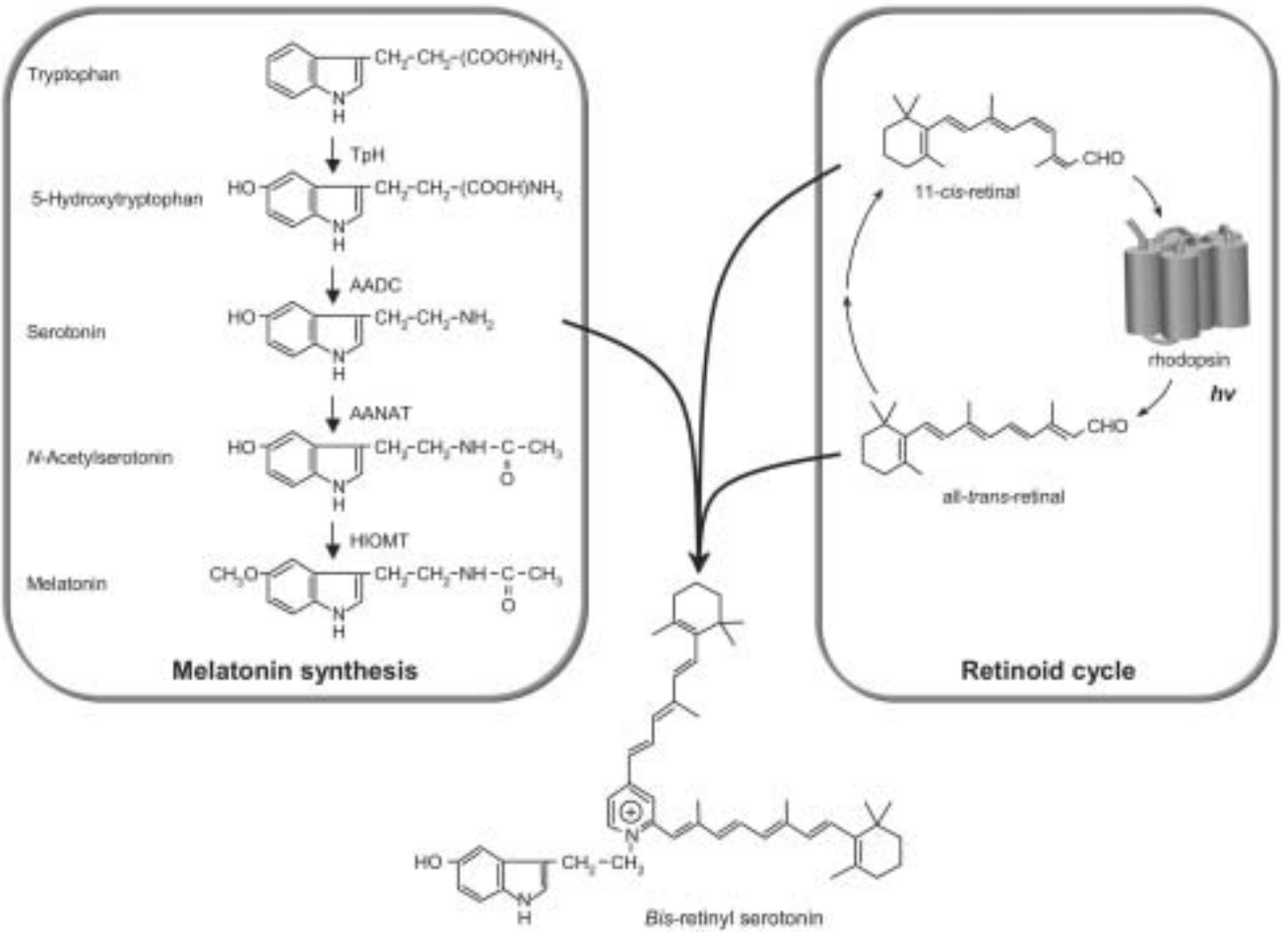

Figure 4. Melatonin synthesis and the retinoid cycle. The interaction of these two pathways is depicted with serotonin and retinal reacting to form *bis*-retinyl serotonin. TpH, tryptophan hydroxylase; AADC, aromatic amino acid decarboxylase; AANAT, arylalkylamine *N*-acetyltransferase; HIOMT, hydroxyindole-*O*-methyltransferase.

It is argued that the resolution of this conflict was the addition of arylalkylamine *N*-acetyltransferase (AANAT) to the phototransduction set of genes. AANAT broadly acetylates arylalkylamines (Ganguly et al., 2001a, 2001b) in addition to serotonin (Fig. 4). Accordingly, it has the capacity of preventing loss of retinaldehyde via A2AA formation.

The hypothesis further proposes that at a later phase in the evolution, when melatonin became recognized as a signal of darkness, a second arylalkylamine/retinaldehyde conflict developed. As the evolving chordates became increasingly dependent on the melatonin signal, the requirement for a reliable supply of melatonin increased. This led to an increase in the intracellular concentration of serotonin; this generated another conflict because it increased the likelihood that retinaldehyde in the photoreceptor would be depleted through *bis*-retinyl serotonin (A2S) (Fig. 4) formation.

The evolutionary resolution of the serotonin/retinaldehyde conflict was to separate retinaldehyde from serotonin by creation of the pinealocyte, allowing the melatonin factory to continue to evolve in one cell and visual transduction in another—the retinal photoreceptor. Gradually, the pinealocyte lost the ability to detect light, and the retina lost the ability to make melatonin, as seen in primates.

It is notable that although the primate retina does not have the ability to make melatonin (Rodriguez et al., 1994; Bernard et al., 1995; Coon et al., 2002), it still retains AANAT, which may play the ancient role of preventing A2AA formation.

The hypothetical nature of this theory must be kept in mind—dealing with issues of evolution is fraught

with difficulties and uncertainties; events that occurred 500,000 years ago are difficult to document. However, the hypothesis was developed in a conservative manner, within the restrictive framework of available information and relevant knowledge. Moreover, some aspects are amenable to testing.

WHEN WAS THE VERTEBRATE MELATONIN SYNTHESIS PATHWAY ASSEMBLED?

Vertebrate melatonin synthesis is defined by the enzymes known to be essential for the conversion of tryptophan to melatonin (Fig. 4). This includes tryptophan hydroxylase, aromatic amino acid decarboxylase, AANAT, and HIOMT. Melatonin synthesis has been claimed to occur in insects, planaria, and other nonvertebrates (Itoh and Sumi, 1998; Itoh et al., 1999; Hardeland and Poeggeler, 2003). However, the genes encoding the required enzymes have not been characterized, nor has their association with phototransduction been established. It is likely, as discussed below, that the reported enzyme activities represent paralogs, i.e., unrelated or very distantly related genes that evolved in parallel with genes found in vertebrates.

The question of when the vertebrate melatonin synthesis pathway was assembled during the course of evolution can be examined by searching complete genomes for the required enzymes. The earliest chordate whose genome is available is the urochordate *Ciona intestinalis*—a tunicate that is also referred to as a sea squirt. Urochordates share a common chordate ancestor with vertebrates, having branched off from the chordate lineage more than 500,000 years ago (Fig. 2).

The larval form of *Ciona* resembles vertebrates, in that it has a distinct notochord connected to a head-like structure. The head contains a primitive brain and a sensory placode with two sensory structures: one—the ocellus—is photosensitive and contains ciliary photoreceptors that have cytoarchitectural features of vertebrate photoreceptors (Fig. 1; Eakin, 1973; Eakin and Kuda, 1971), including a characteristic vertebrate-like photon-capturing outer segment.

Analysis of the near-complete *Ciona* genome reveals that it contains homologs of many of the phototransduction genes. According to the work by Motoyuki Tsuda and his team in Hyogo, several of these genes are selectively expressed in the *Ciona* ocellus (Kusakabe et al., 2001; Nakagawa et al., 2002; Inada et al., 2003). This and the similar cytoarchitecture of the *Ciona* and vertebrate photoreceptors indicate that they share a common precursor photodetector cell.

Analysis of the melatonin synthesis genes indicates that the first two enzymes in the melatonin pathway—tryptophan hydroxylase and aromatic amino acid decarboxylase—are present in *Ciona* (Iyer et al., 2004). However, AANAT and HIOMT are absent. Accordingly, it is clear that although the photodetector cells of the primitive invertebrate chordate *Ciona* were well developed and vertebrate-like, they did not possess the capacity to make melatonin. On the basis of this, one can suspect that the common urochordate/vertebrate ancestor also lacked these genes.

ACQUISITION OF AANAT AND HIOMT

When and how the genes encoding the vertebrate enzymes dedicated to melatonin synthesis were acquired is not clear. The distribution of AANAT and HIOMT in the "Tree of Life" has been studied in detail by Eugene Koonin and his group at the National Library of Medicine in cooperation with my coworker Steve Coon and myself (Iyer et al., 2004). This has revealed that AANAT and HIOMT genes are present in vertebrates, bacteria, and—in the case of AANAT—fungi. However, they are absent from the branches of the tree of life, including plants, worms, insects, and—as indicated above—*Ciona*. An arylalkylamine *N*-acetyltransferase that is very distantly related to the vertebrate AANAT occurs in *Drosophila melanogaster* and *Caenorhabditis elegans* (Hintermann et al., 1995). However, it is a very distantly related paralog—not a homolog; it evolved from a common ancestral acetyltransferase in parallel with AANAT and many other acetyltransferases in the large acetyltransferase superfamily (Neuwald and Landsman, 1997; Iyer et al., 2004), including histone, aminoglycoside, and diamine acetyltransferases. As previously discussed by Vincent Cassone (Cassone and Natesan, 1997), it would seem most likely that the synthesis of melatonin in nonvertebrates reflects the involvement of a different set of genes encoding the enzymes that convert serotonin to melatonin.

The limited phylogenetic distribution of AANAT and HIOMT may be explained by a rare type of evolutionary event: horizontal gene transfer (HGT) (Fig. 5). The common form of gene transfer is vertical (i.e., parent → offspring). HGT involves transfer from one

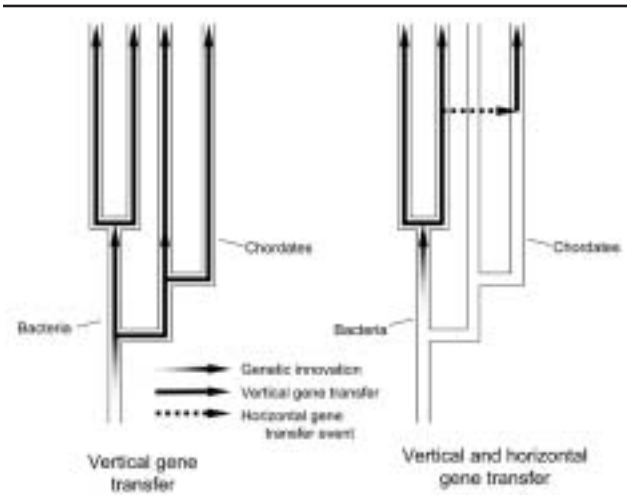

Figure 5. Vertical versus horizontal transfer. An abbreviated evolutionary tree is represented by the branching channels. Genes typically are transferred from parent to offspring in vertical gene transfer. In horizontal gene transfer, genes are transferred from one organism into the germ line (egg or sperm) of another.

organism to the germ line of another and thereby to the recipient's offspring. A well-accepted form of HGT is endosymbiotic transfer, the mechanism apparently responsible for the presence of the chloroplast and mitochondria in cells, as well as for the introduction of many genes that eventually became incorporated into nuclear DNA. HGT among bacteria is now recognized as being common (Doolittle, 1999; Koonin et al., 2001). However, HGT at a later period in evolution involving a bacterial → chordate transfer remains highly controversial and has not been well documented. One example is cellulose synthase, which appears to have been acquired by *Ciona* from bacteria through HGT (Matthysse et al., 2004).

A broader analysis of genes encoding enzymes with functions generally similar to those of AANAT and HIOMT—synthesis of small messengers—has made the interesting observation that many of these have been acquired by vertebrates by scenarios involving HGT. Included in this list are enzymes required for synthesis of histamine, epinephrine, and NO (Iyer et al., 2004). It appears that there was a selective advantage to retain each member of this functional group of genes because of their global importance to the evolution of physiological function, through their roles in the nervous, immune, and neuroendocrine systems.

The mechanism of bacterial → chordate HGT is subject to discussion. It might have occurred during external fertilization. That is, sperm or egg might have received bacterial DNA during fertilization, when exposed to such material present in the dense and biologically rich primordial pond. Insertion of bacterial DNA into germ cell DNA may have been rare, and in most cases, one suspects it had no evolutionary impact because of any one of many reasons. For example, it was inserted out of frame, or at a site where it was not expressed. The most useful type of HGT of AANAT to have maximally affected photoreceptor function would have allowed expression in photoreceptor cells.

Expression in photoreceptor cells might occur if insertion occurred within a gene with regulatory elements that confer photoreceptor-specific expression; this would provide an immediate advantage if the event improved photodetection in some way. Alternatively, the correct transcription factor binding sites to control the tissue-specific expression of these genes might have been acquired through mutation. If so, there would have been a race—between mutations leading to correct expression and mutations that prevented expression of function protein. A reason to favor the first scenario is that it would immediately improve phototransduction and enhance the ability of the recipient organism and its offspring to survive.

The profound impact of two independent successful HGTs of HIOMT and AANAT into the germ line of the vertebrate ancestor cannot be underestimated. These events would globally influence vertebrates because—according to this theory—they would result in the evolution of melatonin signaling and the appearance of the pineal gland.

It is not known yet whether AANAT and HIOMT were absent when cephalochordates—which include amphioxus—emerged from the chordate line. This will become clear when the amphioxus genome becomes available. If AANAT and HIOMT are absent from amphioxus, it would suggest that they were acquired at a later point. This is of importance in considering whether the pinealocyte evolved directly from a single eye—as is present in *Ciona*—or if it was derived from one of sister photoreceptors, as seen in amphioxus. It has been proposed that one of the amphioxus photoreceptors—the lamella organ—is the ancestor of the pinealocyte, based on position in the neural plate, location, and developmental regulation (Glardon et al., 1998). Accordingly, it is possible that AANAT and HIOMT might have become associated with the phototransduction set of genes, which

were expressed in both photodetector sites. That would mean it may have been expressed in both the lamella organ and the frontal organ; in time, however, the retinaldehyde/serotonin conflict would direct the former to become dedicated to melatonin synthesis and the latter to photodetection.

AANAT-ENHANCED ANCESTRAL PHOTORECEPTOR FUNCTION BY PREVENTING LOSS OF RETINALDEHYDE THROUGH A2AA FORMATION

The limited distribution of AANAT and HIOMT in the tree of life raises the issue of why these genes occur in vertebrates—what special pressure selected for their acquisition and, more important, their retention? Similarly, why are these enzyme expressed selectively in ancestral photoreceptors? As first introduced above, the answer comes from the inherent chemical reactivity of retinaldehyde and arylalkylamines.

Retinaldehyde is essential for photodetection (Fig. 3). It binds to opsin in a *cis*-form to create light-sensitive photopigment. Binding occurs via a specific lysine located in the retinaldehyde binding pocket of opsin. Photon capture triggers a *cis*- to *trans*-conformational change that initiates the photochemical transduction cascade. This is accompanied by the release of all-*trans*- retinaldehyde.

The released all-*trans*-retinaldehyde then enters a recycling system—the retinoid cycle—which involves a second compartment system. The first events in the recycling process are binding to an intracellular carrier protein and reduction of the aldehyde to the less reactive all-*trans*-retinol, as catalyzed by all-*trans*-retinaldehyde dehydrogenase. All-*trans*-retinol is then transported from the photoreceptor cell by an interphotoreceptor retinoid binding protein to a second cellular compartment, where it is isomerized to the *cis*-form. In the case of rods, the second compartment is the retinal pigmented epithelial (RPE) cell; in the case of cones, it is the Müeller cell (Mata et al., 2002). In the rod/RPE system, oxidation of *cis*-retinol to *cis*-retinaldehyde by *cis*-retinaldehyde dehydrogenase occurs in the RPE; in the cone/Müeller system, this occurs in the cones.

Many aspects of the two-compartment retinoid cycle are reminders of the selective pressure to preserve retinaldehyde and prevent loss, thereby maintaining a constant source of *cis*-retinaldehyde. Retinaldehyde recycling largely eliminates dependence on the continual replenishment of retinoid from extra-photoreceptor sources. Each molecule of retinaldehyde is reused—ideally continuously—optimizing availability, thereby preventing photobleaching in bright light when flux through the cycle is maximal and also so that the detection of low levels of light is optimized. Specific aspects of the cycle can also be seen to contribute to this conservation. For example, conversion of the aldehyde to the alcohol minimizes loss of retinaldehyde during the retinoid cycle through nonspecific Schiff base reactions with amines. Also, loss through nonspecific binding to proteins and membranes and by diffusion into the circulation is minimized by the association of retinoid with binding proteins. In addition, the two-compartment design of the retinoid cycle allows for *cis*-retinaldehyde to accumulate in the photoreceptor, without inhibiting enzymatic isomerization in the second compartment through feedback inhibition.

These features of the retinoid cycle emphasize the strong selective evolutionary pressure to prevent loss of retinaldehyde and underline the importance that each molecule of retinaldehyde plays in determining photosensitivity. It is notable that Tsuda and his group have provided evidence that a two-compartment system supporting retinoid recycling exists in *Ciona* (Nakashima et al., 2003), consistent with the advanced state of the chordate visual system well before the appearance of vertebrates.

REACTION OF RETINALDEHYDE WITH ARYLALKYLAMINES TO FORM STABLE A2AA ADDUCTS

A2AA adducts belong to a larger group of A2 compounds; the best studied member is a product of retinaldehyde and ethanolamine (A2E), which is composed of two molecules of retinaldehyde and one molecule of ethanolamine bound through a pyridinium ring. The chemical events outlined in Figure 3 are based on the reaction of A2E and retinaldehyde. The identification and synthesis of A2E are the result of pioneering studies by Janet R. Sparrow and Koji Nakanishi and their coworkers (Parish et al., 1998; Sparrow et al., 2003a, 2003b). Their advances reflected an interest in determining the chemical nature of the lipofuscin that accumulates with age in human RPE.

A2E is of current interest because it may play a role in age-related macular degeneration, which leads to blindness (Ben-Shabat et al., 2001, 2002a, 2002b; Sparrow, 2003; Sparrow et al., 2003; Mata et al., 2000). A2E accumulates in the region of the RPE adjacent to the macula region; excessive accumulation is thought to cause macular degeneration. Another A2 family member is A2-opsin, which is formed as a result of the reaction of 2 molecules of retinaldehyde with opsin (Fishkin et al., 2003).

By analogy, one can predict detrimental effects of A2AAs based on current knowledge of A2Es adducts and, from this, understand how they may have played a role in the evolution of the pinealocyte through depletion and toxicity. One is the irreversibility of the pyridinium ring. The stable nature of this ring prevents regeneration of retinaldehyde. As a result, formation of each molecule of A2 compound removes two molecules of retinaldehyde from the retinoid cycle, thereby eroding photosensitivity. In the context of very limited availability of retinoids, continual formation of A2 compounds would be expected to deplete retinaldehyde, and—as seen in other photodetector systems (Zimmerman and Goldsmith, 1971)—photosensitivity would be reduced. In the context of the evolving chordate, in which retinaldehyde may have been limiting and relatively unprotected, this may have had a profound influence.

The second notable characteristic of A2E is related to toxicity. This is the reactivity of the retinoid side chains, which can undergo photooxidation to form epoxide products (Sparrow et al., 2003a, 2003b; Ben-Shabat et al., 2002a, 2002b). Epoxides are highly reactive toward DNA, proteins, and other macromolecules; their formation is accelerated by blue light, and this photochemical event has been implicated in blue light–induced apoptosis of the RPE.

The toxic nature of A2AAs is expected to reflect not only the chemical reactivity of the retinoid side chains but also the chemical nature, instability, and reactivity of the aromatic groups. In the case of A2E, the reactivity of the ethanol side chain is relatively unremarkable; however, the aromatic groups of arylalkylamines will introduce characteristics reflecting the reactivity and nature of the aromatic group and might alter membrane solubility and reactivity through photooxidation of the aromatic ring. In addition, intramolecular interactions between the aromatic rings and retinaldehyde side chains might occur. Accordingly, it is reasonable to suspect that A2AAs have the chemical potential of exhibiting greater toxicity than A2E.

THE SELECTIVE ADVANTAGES OF AANAT AND HIOMT

The A2AA conflict/resolution theory proposes that erosion of photosensitivity by removal of retinaldehyde through the formation of A2AA condensation products threatened the ability of the vertebrate ancestor to survive in the overcrowded and dense Precambrian environment because retinoids were scarce, and xenobiotic and naturally occurring arylalkylamines were abundant. As outlined above, this unavoidable presence of arylalkylamines and their potential reaction with retinaldehyde represented a negative pressure on photodetection—the retinaldehyde/arylalkylamine conflict.

This acquisition of AANAT resolved this conflict because it reduced A2AA formation and prevented retinaldehyde depletion. In this light, AANAT can be grouped with the retinaldehyde protection mechanisms detailed above (conversion of the aldehyde group to the alcohol, binding to proteins) as a molecular mechanism to promote photosensitivity.

The acquisition of HIOMT is likely to have provided a detoxification advantage by stabilizing the hydroxyl group of serotonin, thereby preventing photooxidation leading to ring opening and creation of toxic products. In addition, O-methylation would accelerate elimination because O-methylated products are more soluble in membranes as compared to their hydroxyl-parent compounds. It should also be considered that local high concentrations of *N*-acetylserotonin and melatonin might provide a protective role as antioxidants, which are known to prevent epoxidation of A2E (Sparrow et al., 2003a, 2003b) and, predictably, of A2AAs. Accordingly, like AANAT, the acquisition of HIOMT and retention in the photoreceptor can be viewed as enhancing the detoxification potential of the cell by eliminating arylalkylamines, including serotonin, with a resulting positive influence on photodetection.

This enhanced photosensitivity provided by AANAT and HIOMT is seen as a strong selective advantage because organisms with these genes could

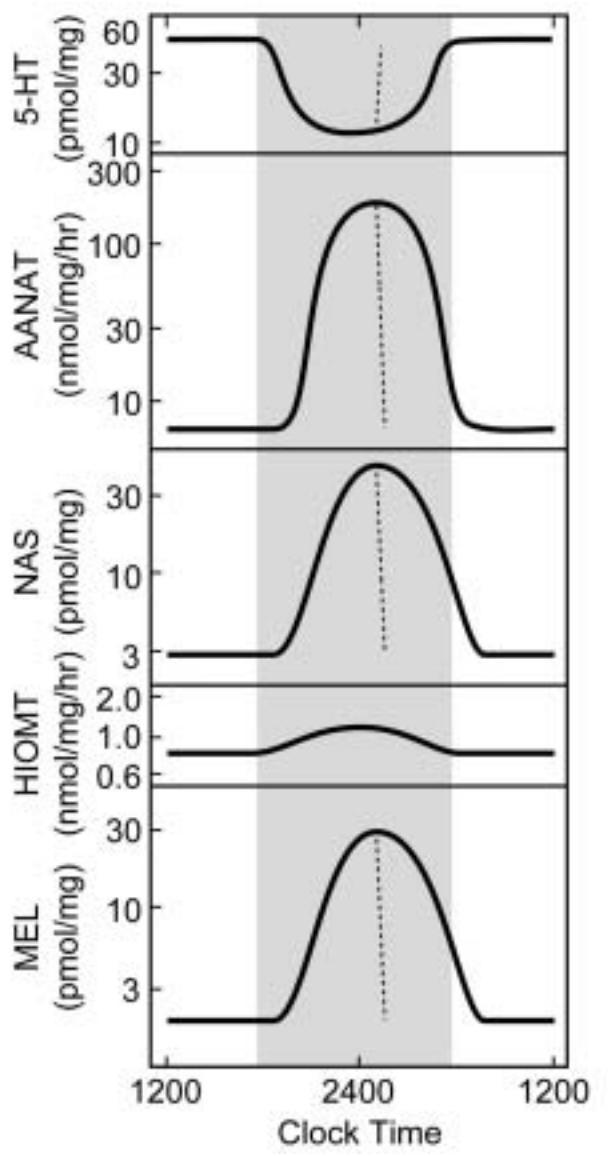

Figure 6. Daily rhythm in the serotonin → melatonin pathway in the pineal gland. Night is represented by the shaded area; the rapid effects of exposure to light at night are depicted by the dashed line. 5-HT, serotonin; AANAT, arylalkylamine *N*-acetyltransferase; NAS, *N*-acetylserotonin; HIOMT, hydroxyindole-O-methyltransferase; MEL, melatonin.

thrive in deeper and darker niches than their competitors lacking these genes: as a result they would have a better chance to survive, reproduce, and populate.

DAILY RHYTHM IN THE MELATONIN PATHWAY

With the acquisition of AANAT and HIOMT, the ancestral chordate had the ability to convert tryptophan to melatonin (Fig. 4). A conserved feature of the melatonin pathway is activation at night, resulting in an increase in melatonin production (Fig. 6). The major dynamic change driving this rhythm is the nocturnal increase in AANAT activity. What pressure selected for this?

By extending the line of thinking regarding the role of AANAT in reducing the loss of retinaldehyde through the formation of A2AA, it appears likely that an increase in AANAT activity would enhance photodetection at a time of the day when it was most important to the ancestral chordate. This would be at night, when a small increase in photosensitivity would have the greatest impact on survival. That is, higher levels of AANAT at night would enhance photosensitivity at night. This may have been the strongest pressure to evolve a nocturnal increase in AANAT activity.

One can envision two possible mechanisms that would generate such an increase in activity at night in photoreceptors; based on what is known about AANAT, one is transcriptional and one is posttranslational. In some cases, transcriptional control is by cyclic AMP through cyclic AMP response element (CRE) binding proteins (Baler et al., 1997); in others, control involves direct links between a circadian clock in the cell and E-box elements in the AANAT promoter (Chong et al., 2000; Chen and Baler, 2000; Munoz and Baler, 2003; Appelbaum et al., 2004). One of these mechanisms might have operated to generate a rhythm in AANAT expression if the bacterial AANAT was inserted into a gene that was expressed at higher levels at night in photoreceptors by either CRE or E-box mechanisms.

Another scenario is that regulation initially was by a posttranslational mechanism similar to that which functions in all vertebrates. This involves cyclic AMP control through phosphorylation of C- and N-terminal cyclic AMP dependent protein kinase (PKA) sites in AANAT and subsequent binding to 14-3-3 proteins (Gastel et al., 1998; Zatz et al., 2000; Falcon et al., 2001; Iuvone et al., 2002; Ganguly et al., 2001a, 2001b; Klein et al., 2002, 2003; Zheng et al., 2003); this prevents degradation and activates the enzyme by increasing the affinity for substrates. It appears that this is the sole regulatory mechanism operating in some vertebrates, including trout, ungulates, and primates; in other cases, it operates in conjunction with transcriptional control mechanisms.

Evolution of the 14-3-3 binding mode of regulation probably occurred after the hypothesized HGT event

because bacterial AANATs do not have C- and N-terminal consensus PKA phosphorylation sites. However, although these elements were absent, it is highly likely that two other critical elements of this regulatory mechanism were in place at the time of acquisition. One is the photic regulation of cyclic nucleotides, which is an element of the phototransduction cascade; the other is the presence of 14-3-3 proteins, which are abundant in all forms of life. These circumstances, together with the selective pressure to increase AANAT activity at night, may have selected for the evolutionary acquisition of consensus PKA motifs by the AANAT gene.

MELATONIN: FROM METABOLIC GARBAGE TO HORMONE OF THE NIGHT

According to this hypothesis, the increase in melatonin at night was—at first—incidental. The status of melatonin changed from metabolic garbage to signal when melatonin became associated with night. This may have occurred through the nonspecific activation of a receptor, which was primarily dedicated to identifying another signaling molecule. This could have been the first step leading to the high-affinity melatonin receptor, which may have evolved through gene duplication and innovation.

Detection of "nighttime" and differentiating night from day have obvious advantages, including coordinating cycles in physiological functions with the dominant feature of the environment—the night/day cycle.

The evolution of melatonin as a hormone requires both synthesis and detection. Without detection, melatonin is garbage. When did the first melatonin receptors evolve?

A detailed analysis of the available genomes by Mark Zylka (unpublished data) has determined that *Ciona* lacks a melatonin receptor gene. Their appearance in chordate evolution may have occurred after cephalochordates emerged from the line leading to vertebrates, based on Eric Bittman's finding that melatonin receptor-like binding is absent from amphioxus (Vernadakis et al., 1998). These receptors are also not detectable in hagfish, although they appear at a later point in the evolution of vertebrates, in the brain of lamprey and skate. If the absence of melatonin receptor binding is a valid indicator of the absence of the melatonin receptor gene, it would appear that melatonin receptors evolved in the chordate lineage between the emergence of hagfish and gnathosomes.

BUILDING A BETTER MELATONIN FACTORY

The "melatonin = night" signal most likely evolved through the interdependent interaction of the evolution of melatonin receptors and of melatonin synthesis. In the first case, the goal was greater and more specific sensitivity; in the second, it was a more consistent and larger melatonin rhythm. Evolution toward a more consistent melatonin signal would require the assembly of a melatonin factory (i.e., a single cell with the enzymes required to convert tryptophan to melatonin).

It is very likely that the continued evolution of this factory within the photoreceptor was impossible because the associated increase in serotonin flux and levels resulted in increased depletion of retinaldehyde through the formation of *bis*-retinyl serotonin (Fig. 4). In addition, the higher levels of serotonin would increase the abundance of photooxidation products in the photoreceptor cells, especially in the high light-intensity environment of the eye.

This conflict between the melatonin pathway and the retinoid cycle (Fig. 4) prevented further coevolution in the same cell.

THE MELATONIN SYNTHESIS/RETINOID CONFLICT RESOLVED: CREATION OF THE PINEALOCYTE

The resolution of this conflict was segregation of the two chemistries into separate cells: one dedicated to melatonin synthesis—the future pinealocyte—and the other dedicated to photodetection—the future retinal photoreceptor.

As pointed out above, it is not clear whether a single photoreceptor organ existed before AANAT was acquired, initiating the formation of two organs, or if two photoreceptor organs already existed, with one evolving toward a pinealocyte and the other toward a retinal photoreceptor cell.

It should be added that the capacity of the retinal photoreceptor to synthesize melatonin from tryptophan has decreased during evolution, in part due to low levels of tryptophan hydroxylase and in

part to low levels of HIOMT; at the highest point, in primates, the retina does not express detectable levels of HIOMT and appears to have lost the capacity to make melatonin (Rodriguez et al., 1994; Bernard et al., 1995; Coon et al., 2002). It does, however, retain high levels of AANAT (Coon et al., 2002).

In response to the question of why the mammalian pinealocyte has lost sensitivity to light, one can guess that the high levels of serotonin in this tissue—0.5 mM in some animals—would lead to high rates of formation of A2AAs. It is unlikely that this would affect melatonin synthesis directly—by a depletion mechanism—because melatonin synthesis is based on extracellular tryptophan, which is abundant. Rather, it seems more likely that high levels of A2AA would accumulate and be toxic. In the case of A2E in the retinal photoreceptor cells, this is minimized by shedding rod outer-segment disks containing A2E. This type of garbage disposal system appears to be essential to the retina. A different evolutionary trend reduced A2AA cytotoxicity in the pineal gland—a deemphasis on phototransduction, which in turn reduced the cellular levels of retinaldehyde; this trend led to the nonphotosensitive mammalian pineal gland.

AANAT IN THE PRIMATE RETINA: FUNCTION AND DRUG TARGET

The level of AANAT in the primate retina is approximately the same as that in pinealocytes—perhaps several-fold higher. In the absence of the capacity to make melatonin, the question of the role of AANAT arises. One possibility is that it is involved in signal transduction, by forming *N*-acetylserotonin, *N*-acetyltryptamine, or both; either might serve as local hormones, acting on local melatonin receptors. Another possibility is that retinal AANAT may play the same protective role it played when first acquired—preventing depletion of retinaldehyde and formation of A2AAs. Arylalkylamines are very likely to be produced in retinal photoreceptor cells through the action of aromatic amino acid decarboxylase, which is ubiquitously present. Therefore, the predicted products of decarboxylation of aromatic amino acid could be produced (i.e., tryptamine, phenylethylamine, and tyramine). At sufficiently high concentrations, they could react with and remove retinaldehyde; this would be minimized by AANAT.

The toxic potential of A2AAs and the possibility that they might contribute to age-related macular degeneration, as does A2E, raises the question of whether the enhancement of AANAT activity might protect against this and reduced AANAT activity might be a permissive. Accordingly, it might be possible, for example, to treat age-related macular degeneration using drugs that promote binding of AANAT to 14-3-3 proteins, thereby protecting and activating the enzyme.

TESTING THE HYPOTHESIS

The best hypotheses are those that not only stimulate thought but also are amenable to testing. Certain elements of this hypothesis are testable. It will be possible, for example, to examine A2AA formation in photodetectors, as well as determine the level of formation and whether enhanced formation reduces photodetection. It is argued that AANAT prevents formation of A2AAs; it will be possible to test this using knockout animals. It is argued that arylalkylamines eroded photosensitivity in the primitive photoreceptor; this could be tested in *Ciona*, which exhibits photoresponses mediated by the ocellus. It will also be possible to test whether AANAT and HIOMT prevent this in *Ciona* by transfecting these genes using established promoters that direct expression in photoreceptor cells (Inada et al., 2003). The idea that AANAT and HIOMT were introduced by HGT will be continually tested as new complete genome sequences become available—the finding of these genes in genomes of plants, worms, and insects would erode the HGT argument.

This hypothesis argues for a previously unappreciated and unrecognized role of AANAT—preventing formation of A2AA compounds. It is possible that this remains the central role of the enzyme in the primate retina—prevention of the formation of A2AAs and their toxic products. It will be important to examine this by determining if A2AAs are present in the primate retina and if there is an association between age-related macular degeneration and AANAT levels through pharmacological manipulation. Another approach would be to determine if there are individual differences in AANAT activity, perhaps genetically determined, and if these are associated with a higher risk for age-related macular degeneration.

ACKNOWLEDGMENTS

I wish to express appreciation to Mark Zylka for searching for *Ciona*melatonin receptors and to Steven Coon and Ken Kirk for allowing me to include reference to our unpublished results on the formation of A2AAs. Appreciation is also expressed to Marty Zatz, Mike Iuvone, Ruben Baler, and Jack Falcon for their critical reading of the manuscript and constructive comments

REFERENCES

Appelbaum L, Toyama R, Dawid IB, Klein DC, Baler R, and Gothilf Y (2004) Zebrafish serotonin *N*-acetyltransferase-2 gene regulation: Pineal-restrictive downstream module contains a functional E-box and three photoreceptor conserved elements. Mol Endocrinol 5:1210-1221.

Araki M (1992) Cellular mechanism for norepinephrine suppression of pineal photoreceptor-like cell differentiation in rat pineal cultures. Dev Biol 149:440-447.

Araki M, Fukada Y, Shichida Y, Yoshizawa T, and Tokunaga F (1992) Differentiation of both rod and cone types of photoreceptors in the in vivo and in vitro developing pineal glands of the quail. Dev Brain Res 65:85-92.

Araki M and Tokunaga F (1990) Norepinephrine suppresses both photoreceptor and neuron-like properties expressed by cultured rat pineal glands. Cell Differ Dev 31:129-135.

Araki M, Watanabe K, Tokunaga F, and Nonaka T (1988) Phenotypic expression of photoreceptor and endocrine cell properties by cultured pineal cells of the newborn rat. Cell Differ Dev 25:155-163.

Axelrod J, Quay WB, and Baker PC (1965) Enzymatic synthesis of the skin lightening agent, melatonin, in amphibians. Nature 208:386.

Babila T, Schaad NC, Simonds WF, Shinohara T, and Klein DC (1992) Development of MEKA (phosducin), G beta, G gamma, and S-antigen in the rat pineal gland and retina. Brain Res 585:141-148.

Baler R, Covington S, and Klein DC (1997) The rat arylalkylamine *N*-acetyltransferase gene promoter: cAMP activation via a cAMP-responsive element-CCAAT complex. J Biol Chem 272:6979-6985.

Bastianelli E and Pochet R (1994) Calbindin D28k, calretinin, and recoverin immunoreactivities in developing chick pineal gland. J Pineal Res 17:103-111.

Ben-Shabat S, Itagaki Y, Jockusch S, Sparrow JR, Turro NJ, and Nakanishi K (2002a) Formation of a nonaoxirane from A2E, a lipofuscin fluorophore related to macular degeneration, and evidence of singlet oxygen involvement. Angew Chem Int Ed Engl 41:814-817.

Ben-Shabat S, Parish CA, Hashimoto M, Liu J, Nakanishi K, and Sparrow JR (2001) Fluorescent pigments of the retinal pigment epithelium and age-related macular degeneration. Bioorg Med Chem Lett 11:1533-1540.

Ben-Shabat S, Parish CA, Vollmer HR, Itagaki Y, Fishkin N, Nakanishi K, and Sparrow JR (2002b) Biosynthetic studies of A2E, a major fluorophore of retinal pigment epithelial lipofuscin. J Biol Chem 277:7183-7190.

Bernard M, Dinet V, and Voisin P (2001) Transcriptional regulation of the chicken hydroxyindole-O-methyltransferase gene by the cone-rod homeobox-containing protein. J Neurochem 79:248-257.

Bernard M, Donohue SJ, and Klein DC (1995) Human hydroxyindole-O-methyltransferase in pineal gland, retina and Y79 retinoblastoma cells. Brain Res 696:37-48.

Blackshaw S and Snyder SH (1997) Parapinopsin, a novel catfish opsin localized to the parapineal organ, defines a new gene family. J Neurosci 17:8083-8092.

Cahill GM, Grace MS, and Besharse JC (1991) Rhythmic regulation of retinal melatonin: Metabolic pathways, neurochemical mechanisms, and the ocular circadian clock. Cell Mol Neurobiol 11:529-560.

Cassone VM and Natesan AK (1997) Time and time again: The phylogeny of melatonin as a transducer of biological time. J Biol Rhythms 12:489-497.

Chen S, Wang QL, Nie Z, Sun H, Lennon G, Copeland NG, Gilbert DJ, Jenkins NA, and Zack DJ (1997) Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. Neuron 19:1017-1030.

Chen W and Baler R (2000) The rat arylalkylamine *N*-acetyltransferase E-box: Differential use in a master vs. a slave oscillator. Brain Res Mol Brain Res 81:43-50.

Chong NW, Bernard M, and Klein DC (2000) Characterization of the chicken serotonin *N*-acetyltransferase gene: Activation via clock gene heterodimer/E box interaction. J Biol Chem 275:32991-32998.

Collin JP (1968) Pluralité des "photorécepteurs" dans l'é piphyse de Lacerta. C R Acad Sci (Paris) D 267, 1047-1050. Cell Tissue Res 240:693-700.

Collin JP (1971) Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. In *The Pineal Gland*, Wolstenholme GEW and Knight J., eds, pp 79-120, Churchill Livingstone, London.

Collin JP (1977) La rudimentation des photorécepteurs dans l'organe pineal des vertébres. In *Mécanismes de la rudimentation des organes chez les embryons de vertébrés*, vol. 266, Raynaud A., ed, pp 393-407, Paris, Coll Int CNRS.

Collin JP, Brisson P, Falcón J, and Voisin P (1986a) Multiple cell types in the pineal: Functional aspects. In *Pineal and Retinal Relationships*, O'Brien PJ, and Klein DC, eds, pp 15-32, Orlando, FL, Academic Press.

Collin JP, Mirshahi M, Brisson P, Falcon J, Guerlotte J, and Faure JP (1986b) Pineal-retinal molecular relationships: Distribution of "S-antigen" in the pineal complex. Neuroscience 19:657-666.

Collin JP and Oksche A (1981) Structural and functional relationships in the nonmammalian pineal organ. In *The Pineal Gland: Vol. 1. Anatomy and Biochemistry*, RJ Reiter, ed, pp 27-67, CRC Press, Boca Raton, FL.

Collin JP, Voisin P, Falcon J, Faure JP, Brisson P, and Defaye JR (1989) Pineal transducers in the course of evolution: Molecular organization, rhythmic metabolic activity and role. Arch Histol Cytol 52(Suppl.):441-449.

Coon SL, Del Olmo E, Young WS III, and Klein DC (2002) Melatonin synthesis enzymes in *Macaca mulatta*: Focus on arylalkylamine *N*-acetyltransferase (EC 2.3.1.87). J Clin Endocrinol Metab 87:4699-4706.

Donoso LA, Merryman CF, Edelberg KE, Naids R, and Kalsow C (1985) S-antigen in the developing retina and pineal gland: A monoclonal antibody study. Invest Ophthalmol Vis Sci 26:561-567.

Doolittle WF (1999) Lateral genomics. Trends Cell Biol 9:M5-M8.

Eakin RM (1973) *The Third Eye*, University of California Press, Berkeley.

Eakin RM and Kuda A (1971) An ultrastructure of sensory receptors in Ascidian tadpoles. Z Zellforsch Mikrosk Anat 112:287-312.

Ekstrom P and Meissl H (2003) Evolution of photosensory pineal organs in new light: The fate of neuroendocrine photoreceptors. Philos Trans R Soc Lond B Biol Sci 358:1679-1700.

Falcon J (1984) Photosensitivity and biosynthesis of indole compounds in the cells of the receptor line of the pineal organ of the pike. Ophthalmic Res 16:123-128.

Falcon (1999) Cellular circadian clocks in the pineal. Prog. Neurobiol 58:121-162.

Falcon J, Galarneau KM, Weller JL, Ron B, Chen G, Coon SL, and Klein DC (2001) Regulation of arylalkylamine *N*-acetyltransferase-2 (AANAT2, EC 2.3.1.87) in the fish pineal organ: Evidence for a role of proteosomal proteolysis. Endocrinol 142:1804-1813.

Falcon J, Gothilf Y, Coon SL, Boeuf G, and Klein DC (2003) Genetic, temporal, and developmental differences between melatonin rhythm generating systems in the teleost fish pineal organ and retina. J Neuroendocrinol 15:378-382.

Fishkin N, Jang YP, Itagaki Y, Sparrow JR, and Nakanishi K (2003) A2-rhodopsin: A new fluorophore isolated from photoreceptor outer segments. Org Biomol Chem 1:1101-1105.

Flight WF, Mans D, and Balemans MG (1983) Methoxyindole synthesis in the retina of the frog (*Rana esculenta*) during a diurnal period. J Neural Transm 58(3-4):223-230.

Furukawa T, Morrow EM, Li T, Davis FC, and Cepko CL (1999) Retinopathy and attenuated circadian entrainment in Crx-deficient mice. Nat Genet 23:466-470.

Gamse JT, Shen YC, Thisse C, Thisse B, Raymond PA, Halpern ME, and Liang JO (2002) Otx5 regulates genes that show circadian expression in the zebrafish pineal complex. Nat Genet 30:117-121.

Ganguly S, Coon SL, and Klein DC (2002) Control of melatonin synthesis in the mammalian pineal gland: The critical role of serotonin acetylation. Cell Tissue Res 309:127-137.

Ganguly S, Gastel JA, Weller JL, Schwartz C, Jaffe H, Namboodiri MA, Coon SL, Hickman AB, Rollag M, Obsil T, et al. (2001a) Role of a pineal cAMP-operated arylalkylamine *N*-acetyltransferase/14-3-3-binding switch in melatonin synthesis. Proc Natl Acad Sci USA 98:8083-8088.

Ganguly S, Mummaneni P, Steinbach PJ, Klein DC, and Coon SL (2001b) Characterization of the *Saccharomyces cerevisiae* homolog of the melatonin rhythm enzyme arylalkylamine *N*-acetyltransferase (EC 2.3.1.87). J Biol Chem 276:47239-47247.

Gastel JA, Roseboom PH, Rinaldi PA, Weller JL, and Klein DC (1998) Melatonin production: Proteasomal proteolysis in serotonin *N*-acetyltransferase regulation. Science 279:1358-1360.

Glardon S, Holland LZ, Gehring WJ, Holland ND (1998) Isolation and developmental expression of the amphioxus Pax-6 gene (AmphiPax-6): Insights into eye and photoreceptor evolution. Development 125:2701-10.

Hardeland R and Poeggeler B (2003) Non-vertebrate melatonin. J Pineal Res 34:233-241.

Hintermann E, Jeno P, and Meyer UA (1995) Isolation and characterization of an arylalkylamine *N*-acetyltransferase from *Drosophila melanogaster*. FEBS Lett 375:148-150.

Ho AK, Somers RL, and Klein DC (1986) Development and regulation of rhodopsin kinase in rat pineal and retina. J Neurochem 46:1176-1179.

Inada K, Horie T, Kusakabe T, and Tsuda M (2003) Targeted knockdown of an opsin gene inhibits the swimming behaviour photoresponse of ascidian larvae. Neurosci Lett 347:167-170.

Itoh MT, Shinozawa T, and Sumi Y (1999) Circadian rhythms of melatonin synthesizing enzyme activities and melatonin levels in planarians. Brain Res 830:165-173.

Itoh MT and Sumi Y (1998) Melatonin and serotonin *N*-acetyltransferase activity in developing eggs of the cricket *Gryllus bimaculatus*. Brain Res 781:90-99.

Iuvone PM, Brown AD, Haque R, Weller J, Zawilska JB, Chaurasia SS, Ma M, and Klein DC (2002) Retinal melatonin production: Role of proteasomal proteolysis in circadian and photic control of arylalkylamine *N*-acetyltransferase. Invest Ophthalmol Vis Sci 43:564-572.

Iuvone PM, Chong NW, Bernard M, Brown AD, Thomas KB, and Klein DC (1999) Melatonin biosynthesis in chicken retina: Regulation of tryptophan hydroxylase and arylalkylamine *N*-acetyltransferase. Adv Exp Med Biol 460:31-41.

Iyer LM, Aravind L, Coon SL, Klein DC, and Koonin EV (2004) Evolution of cell-cell signaling in animals: Did late horizontal gene transfer from bacteria play a role? Trends Genet 20; in press. Published electronically 28 May 2004 at http://www.sciencedirect.com/science/journal/01689525.

Kalsow CM and Wacker WB (1977) Pineal reactivity of anti-retina sera. Invest Ophthalmol Vis Sci 16:181-184.

Kappers JA and JP Schadé, eds (1965) *Structure and Function of the Epiphysis Cerebri*, Amsterdam, Elsevier.

Klein DC, Baler R, Roseboom PH, Weller JL, Bernard M, Gastel JA, Zatz M, Iuvone PM, Begáy V, Falcón J, et al. (1998) The molecular basis of the pineal melatonin rhythm: Regulation of serotonin *N*-acetylation. In *Handbook of Behavioral State Control: Cellular and Molecular Mechanisms*, Lydic R and Baghdoyan H, eds, pp 45-59, Boca Raton, FL, CRC Press.

Klein DC, Coon SL, Roseboom PH, Weller JL, Bernard M, Gastel JA, Zatz M, Iuvone PM, Rodriguez IR, Begay V,

et al. (1997) The melatonin rhythm-generating enzyme: Molecular regulation of serotonin *N*-acetyltransferase in the pineal gland. Recent Prog Horm Res 52:307-357.

Klein DC, Ganguly S, Coon S, Weller JL, Obsil T, Hickman A, and Dyda F (2002) 14-3-3 Proteins and photoneuroendocrine transduction: Role in controlling the -*O*-rhythm in melatonin. Biochem Soc Trans 30:365-373.

Klein DC, Ganguly S, Coon SL, Shi Q, Gaildrat P, Morin F, Weller JL, Obsil T, Hickman A, and Dyda F (2003) 14-3-3 Proteins in pineal photoneuroendocrine transduction: How many roles? Neuroendocrinology 15:370-377.

Koonin EV, Makarova KS, and Aravind L (2001) Horizontal gene transfer in prokaryotes: Quantification and classification. Annu Rev Microbiol 55:709-742.

Korf HW (1994) The pineal organ as a component of the biological clock: Phylogenetic and ontogenetic considerations. Ann NY Acad Sci 719:13-42.

Korf HW (1999) Evolution of melatonin producing pinealocytes. Adv Exp Med Biol 460:17-29.

Korf HW, Moller M, Gery I, Zigler JS, and Klein DC (1985) Immunocytochemical demonstration of retinal S-antigen in the pineal organ of four mammalian species. Cell Tissue Res 239:81-85.

Korf HW, White BH, Schaad NC, and Klein DC (1992) Recoverin in pineal organs and retinae of various vertebrate species including man. Brain Res 595:57-66.

Kusakabe T, Kusakabe R, Kawakami I, Satou Y, Satoh N, and Tsuda M (2001) Ci-opsin1, a vertebrate-type opsin gene, expressed in the larval ocellus of the ascidian *Ciona intestinalis*. FEBS Lett 506:69-72.

Mano H, Kojima D, and Fukada Y (1999) Exo-rhodopsin: A novel rhodopsin expressed in the zebrafish pineal gland. Brain Res Mol Brain Res 73:110-118.

Mata NL, Radu RA, Clemmons RC, and Travis GH (2002) Isomerization and oxidation of vitamin A in cone-dominant retinas: A novel pathway for visual-pigment regeneration in daylight. Neuron 36:69-80.

Mata NL, Weng J, and Travis GH (2000) Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. Proc Natl Acad Sci USA 97:7154-7159.

Matthysse AG, Deschet K, Williams M, Marry M, White AR, and Smith WC (2004) A functional cellulose synthase from ascidian epidermis. Proc Natl Acad Sci USA 101:986-991.

Max M, McKinnon PJ, Seidenman KJ, Barrett RK, Applebury ML, Takahashi JS, and Margolskee RF (1995) Pineal opsin: A nonvisual opsin expressed in chick pineal. Science 267:1502-1506.

Munoz E and Baler R (2003) The circadian E-box: When perfect is not good enough. Chronobiol Int 20:371-388.

Nakagawa M, Orii H, Yoshida N, Jojima E, Horie T, Yoshida R, Haga T, and Tsuda M (2002) Ascidian arrestin (Ci-arr), the origin of the visual and nonvisual arrestins of vertebrate. Eur J Biochem 269:5112-5118.

Nakashima Y, Kusakabe T, Kusakabe R, Terakita A, Shichida Y, and Tsuda M (2003) Origin of the vertebrate visual cycle: Genes encoding retinal photoisomerase and two putative visual cycle proteins are expressed in whole brain of a primitive chordate. J Comp Neurol 460:180-190.

Neuwald AF and Landsman D (1997) GCN5-related histone *N*-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Trends Biochem Sci 22:154-155.

Nishida A, Furukawa A, Koike C, Tano Y, Aizawa S, Matsuo I, and Furukawa T (2003) Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development. Nat Neurosci 6:1255-1263.

O'Brien PJ and Klein DC, eds (1986) *Pineal and Retinal Relationships*, Orlando, FL, Academic Press.

Okano T, Yoshizawa T, and Fukada Y (1994) Pinopsin is a chicken pineal photoreceptive molecule. Nature 372: 94-97.

Oksche A (1965) Survey of the development and comparative morphology of the pineal organ. In *Structure and Function of the Epiphysis Cerebri*, Kappers JA and Schadé JP, eds, pp 3-30, Amsterdam, Elsevier.

Oksche A (1971) Sensory and glandular elements of the pineal organ. In *The Pineal Gland*, Wolstenholme GEW and Knight J, eds, pp 127-146, London, Churchill Livingstone.

Oksche A (1983) Aspects of evolution of the pineal organ. In *The Pineal Gland and Its Endocrine Role*, Axelrod J, Fraschini F, and Velo GP, eds, pp 15-35, New York, Plenum.

Oksche A (1984) Evolution of the pineal complex: Correlation of structure and function. Ophthalmic Res 16:88-95.

Oksche A (1991) The development of the concept of photoneuroendocrine systems: Historical perspective. In Klein DC, Moore RY, and Reppert SM (eds), Suprachiasmatic Nucleus: The Mind's Clock. New York, Oxford University Press.

Parish CA, Hashimoto M, Nakanishi K, Dillon J, and Sparrow J (1998) Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium. Proc Natl Acad Sci USA 95(25):14609-14613.

Reig JA, Yu L, and Klein DC (1990) Pineal transduction. Adrenergic → cyclic AMP-dependent phosphorylation of cytoplasmic 33-kDa protein (MEKA) which binds beta gamma-complex of transducin. J Biol Chem 265:5816-5824.

Rodrigues MM, Hackett J, Gaskins R, Wiggert B, Lee L, Redmond M, and Chader GJ (1986) Interphotoreceptor retinoid-binding protein in retinal rod cells and pineal gland. Invest Ophthalmol Vis Sci 27:844-850.

Rodriguez IR, Mazuruk K, Schoen TJ, and Chader GJ (1994) Structural analysis of the human hydroxyindole-O-methyltransferase gene: Presence of two distinct promoters. J Biol Chem 269:31969-31977.

Schaad NC, Shinohara T, Abe T, and Klein DC (1991) Photoneural control of the synthesis and phosphorylation of pineal MEKA (phosducin). Endocrinology 129:3289-3298.

Somers RL and Klein DC (1984) Rhodopsin kinase activity in the mammalian pineal gland and other tissues. Science 226:182-184.

Sparrow JR (2003) Therapy for macular degeneration: Insights from acne. Proc Natl Acad Sci USA 100:4353-4354.

Sparrow JR, Fishkin N, Zhou J, Cai B, Jang YP, Krane S, Itagaki Y, and Nakanishi K (2003a) A2E, a byproduct of the visual cycle. Vision Res 43:2983-2990.

Sparrow JR, Nakanishi K, and Parish CA (2000) The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. Invest Ophthalmol Vis Sci 41:1981-1989.

Sparrow JR, Vollmer-Snarr HR, Zhou J, Jang YP, Jockusch S, Itagaki Y, and Nakanishi K (2003b) A2E-epoxides damage DNA in retinal pigment epithelial cells: Vitamin E and other antioxidants inhibit A2E-epoxide formation. J Biol Chem 278:18207-18213.

Tosini G and Fukuhara C (2003) Photic and circadian regulation of retinal melatonin in mammals. J Neuroendocrinol 15:364-369.

van Veen T, Elofsson R, Hartwig HG, Gery I, Mochizuki M, Cena V, and Klein DC (1986a) Retinal S-antigen: Immunocytochemical and immunochemical studies on distribution in animal photoreceptors and pineal organs. Exp Biol 45:15-25.

van Veen T, Ostholm T, Gierschik P, Spiegel A, Somers R, Korf HW, and Klein DC (1986b) alpha-Transducin immunoreactivity in retinae and sensory pineal organs of adult vertebrates. Proc Natl Acad Sci USA 83:912-916.

Vernadakis AJ, Bemis WE, and Bittman EL (1998) Localization and partial characterization of melatonin receptors in amphioxus, hagfish, lamprey, and skate. Gen Comp Endocrinol 110:67-78.

Zatz M, Gastel JA, Heath JR III, and Klein DC (2000) Chick pineal melatonin synthesis: Light and cyclic AMP control abundance of serotonin *N*-acetyltransferase protein. J Neurochem 74:2315-2321.

Zheng W, Zhang Z, Ganguly S, Weller JL, Klein DC, and Cole PA (2003) Cellular stabilization of the melatonin rhythm enzyme induced by nonhydrolyzable phosphonate incorporation. Nat Struct Biol 10:1054-1057.

Zimmerman BL and Tso MOM (1975) Morphological evidence of photoreceptor differentiation of pinealocytes in the neonatal rat. J Cell Biol 66:60-75.

Zimmerman WF and Goldsmith TH (1971) Photosensitivity of the circadian rhythm and of visual receptors in carotenoid-depleted *Drosophila*. Science 171:1167-1169.